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Genetic Distance of Four Strains of Guppy Fish (*Poecilia reticulata*) Using RAPD PCR Method

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Guppies are one type of organism that can live well, especially in the tropics. The kinship between species that have genetic similarities can be identified through genotypic mapping, one of which is molecular analysis using. RAPD method with PCR technique. This study aims to determine the genetic relationship of four guppy poecilia reticulata strains, respectively are Albino Full Red (AFP), Brazilian Fan Tail (BFT), Koi Guppy Tuxedo (KGT), and Platinum Red Tail Big Ears (PRTB) with the RAPD-PCR method. The genetic relationship data obtained is used as a guide for mating between the four strains. This research was conducted from September 2020 to November 2020. The process were carried out at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University and the Central Laboratory, Padjadjaran University. OPA-03 primer (AGTCAGCCAC) is used as a standard parameter to interpret genetic diversity among the four guppy strains. Based on the results, amplification with primer OPA-03 visualized 21 bands consisting of seven polymorphic bands and 13 monomorphic bands. The results of the phylogenetic tree showed that there were two groups. The first group is AFR and BFT a similarity index of 69.5%. The second group is KGT and PRTB a similarity index of 71.5%.

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Keywords: Dendrogram; guppy; genetic distance; RAPD- PCR.

1. INTRODUCTION

Indonesia has the largest potential to produce ornamental fish in the world. One of a great demand is guppy fish (*Poecilia reticulata*), the guppy fish also know as the Million fish or the Rainbow Fish [1]. According to [2] there are more than 150 species identified and can be cultivated.

The attraction of guppy has beautiful color variations, various patterns on the body and tail, and various tail shapes [3]. According to [4, 5], The highest demand is a male fish, because male fish have bright colors, small and slender body shapes, and long and wide tail shapes. This causes male fish to be more economically valuable.

The number of guppy fish enthusiasts makes cultivators cross to get new variations. Crosses are carried out only by looking at the morphology of the male and female broodstock because it will increase the risk of mating which will result in defective offspring.

The study used four strains of male guppy whose genetic distance was unknown. The study was conducted to obtain information to facilitate crossing between these guppy strains.

Determination of genetic distance can be determined by using the Polymerase Chain Reaction (PCR) technique with Random Amplified of Polymorphic DNA (RAPD) method. The RAPD method is used to detect DNA polymorphism that are used as genetic markers and determine kinship relationships in various plants and animals [6, 7]. According to [8], the advantage of the RAPD method are relatively simple, requiring a small quantity of DNA (5 -25 ng of DNA) in each PCR chain. Another advantage is that RAPD has criteria as an ideal marking system because of its high polymorphic, easy and fast, and cost lest [9, 10, 11].

The results of the data obtained from the RAPD-PCR method were then processed using the NTSYS (Numerical Taxonomy and Multivariate Analysis System) program to get a dendrogram tree or kinship tree in the test sample and then analyzed for genetic kinship [12, 13].

Therefore, a study was carried out to determine the genetic distance of guppies so that the results of kinship data could be used as a reference for spawning and could produce different variations of fish.

The guppy strains are Albino Full Red (AFR), Brazilian Fantail (BFT), Koi Guppy Tuxedo (KGT) and Platinum Red Tail Big Ears (PRTB).

2. MATERIALS AND METHODS

This research was conducted from September 2020 to November 2020. The process of DNA isolation, DNA amplification, DNA isolation electrophoresis, and DNA amplification were carried out at the Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University. Meanwhile, for the calculation of DNA purity at the Central Laboratory, Padjadjaran University.

The AFP, BFT, and PRTB strains obtained from Cilengkrang District Bandung, and the KGT obtained from Tangerang, Banten.

The research method used an explorative method without using experimental design and qualitatively. The samples used were guppy caudal fins from four different strains. Determination of genetic diversity using the Random Amplified Polymorphic DNA (RAPD) - Polymerase Chain Reaction (PCR) method and with the help of two universal primers (OPA-02 and OPA-03). From these methods, monomorphic and polymorphic bands will appear, [14] which are then processed using the NTSYS program (Numerical Taxonomy and Multivariate Analysis System) and produce a dendrogram tree.

3. PROCEDURES

Research procedures include:

3.1 DNA Isolation

DNA isolation in this study used the Genomic DNA Purification Kit (Promega). 10 mg of caudal fin was added to a 1.5 µl Eppendorf tube and crushed until smooth, added 300 µl of Nuclei lysis solution, homogenized with a vortex for 10 seconds, and incubated at 65ºC for 30 minutes. 1.5 µl of RNAse was added, inverted 2-5 times, incubated at 37ºC for 30 minutes, and cooled for 5 minutes at room temperature. Precipitation solution protein of added 100 mL , homogenized with a vortex for 10 seconds, and centrifuged at 13,000 rpm for 4 minutes. The supernatant was

transferred to a new microtube, added 300 µl of isopropanol, and centrifuged at 13,000 rpm for 1 minute. The supernatant was discarded, then added 300 µl of ethanol, and centrifuged again at 13,000 rpm for 1 minute. Remove the ethanol and the pellets were dried for 15 minutes. 50 µl of TE solution was added and incubated at 65ºC for 60 minutes. The tubes are stored at -20ºC.

3.2 Calculation of DNA Purity

DNA purity quantification is a quantitative test of DNA using a Multimode Reader Infinite 200 PRO NanoQuant spectrophotometer at a wavelength of 260 and 280 nm. Added 2 µl of DNA isolation and 2 µl of nuclease-free water into the cuvette, then the absorbance was adjusted on the instrument with a wavelength of 260 nm and 280 nm. The results are recorded and counted. Result of Dna purity used [15]:

$$\text{DNA purity} = A_{260} X \frac{50ng}{\mu l} dilution\ factor$$

$$Dilution\ factor\ = 50\ dultion\ factor$$

$$DNA\ purity: \frac{260}{280}$$

A_{260} : Absorbance value at (λ) 260 nm.
50 : A solution with an absorbance value of 1.0 is equivalent to 50 g of double-stranded DNA per ml.

3.3 DNA Amplification

DNA amplification procedure was carried out by added 12.5 µl of GoTaq Green Master Mix (Promega), 2 µl of Genomic DNA, 1.3 µl of primer, and 9.2 µl of nuclease-free water to 0.2 ml microtube and homogenized with a vortex for 10 seconds. Microtubes inserted into the PCR machine with the following programs: pre-denaturation (94ºC, 2 minutes, 1 cycle), denaturation (94ºC, 1 minute, 45 cycles), annealing (36ºC, 1 minute, 45 cycles), extension (72ºC, 2 minutes). , 45 cycles), final extension (72ºC, 10 minutes, 1 cycle), hold (4ºC, 3 minutes, 1 cycle) [13, 16].

3.4 Electrophoresis

The results of DNA isolation and amplification results were tested qualitatively by electrophoresis with agarose gel. The procedure was carried out to make 1% agarose concentration by adding 0.8 g of agarose powder and 80 ml of 1x TAE solution into an Erlenmeyer, then put in the microwave for 3 minutes, added 0.8 µl of gel read gel, and then homogenized. The solution is cooled and poured into an agarose mold equipped with a comb, waiting for 20-30 minutes until frozen. Added 1x TAE Buffer poured with sub-marine technique. The first pit sample was filled with 2 µl DNA Ladder 1kb and 2 µl loading dye. The next pit was filled with 4 µl of DNA template and 2 µl of loading dye. The electrophoresis tank was given electricity at 75V for 45 minutes (for DNA isolation results) and 90 minutes (for DNA amplification results).

3.5 DATA Analysis

Data analysis was carried out qualitatively and quantitatively. Quantitative data is to get from the calculation of DNA purity. Qualitative data get from visualized and un-visualized bands on agarose gel, then qualitative data was made in the form of a binary matrix with the help of Corel Draw and Microsoft Excel 2003 and processed using the NTSYS-PC program to generate a dendrogram tree from four samples of guppy fish strains to reveal genetic distance.

4. RESULTS AND DISCUSSION

4.1 DNA Isolation Result

The first test is a qualitative test by looking at the appearance of the band on the agarose. The results of DNA isolation showed that the DNA produced was of good enough quality, there are DNA bands on the agarose gel it can be seen in Fig. 1.

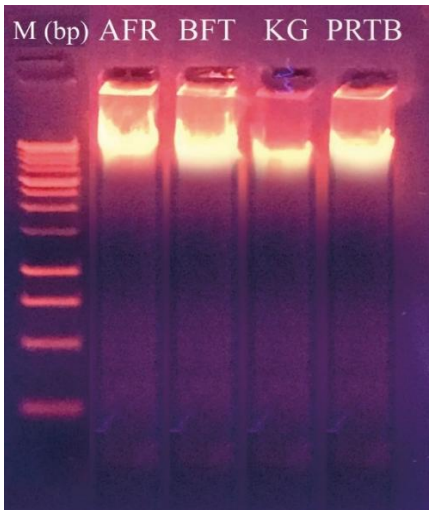

Fig. 1. Electrophoresis of DNA isolation result
Note: bp = base pair; M = Marker 1 Kb; AFP=Albino Full Red; BFT= Brazilian Fan Tail; KGT=Koi Guppy Tuxedo; PRTB=Platinum Red Tail Big Ears

In this study, the DNA isolation process used the Wizard Genomic DNA Purification Kit (Promega) which has been well tested and is capable of producing thick or large genomic DNA, very clearly formed genomic DNA bands [17, 18]. Caudal fin used, because the caudal fin is easily destroyed in the research process so it will be easy to get DNA samples [13].

In the next stage, a quantitative test is carried out. The testing phase is assisted by a spectrophotometer to determine the level of concentration and purity level of genomic DNA by paying attention to the values at Abs260 nm and Abs280 nm. The purity level of pure DNA isolates has a ratio value of 1.8-2.0. If the ratio value is lower than 1.8, there are protein or phenol contaminants, but if the ratio value is higher than 2.0 there are RNA contaminants [19, 20].

The calculation of the concentration and purity of DNA in this study used the Multimode Reader Infinite 200 PRO NanoQuant spectrophotometer. The results obtained from four samples can see in table 4. Three strains AFR, BFT, and PRTB had a purity value of 1.85; 1.92; 1.89 where the range of numbers corresponds to pure DNA between 1.8-2.0. Meanwhile, the KGT fish strain sample has a purity value of 1.78 where this value is not fit the range of pure DNA values.

4.2 DNA Amplification

In the DNA amplification stage, two universal primers were used, namely the OPA-02 primer and the OPA-03 primer. Among the two primers, the OPA-03 primer produced more bands, so the PCR produk with the OPA-03 primer was electrophoresed. The results of electrophoresis can see in Fig. 2.

According to [21] the base composition of each universal primer is different, so the amplified fragments are different. According to [13], the number and size of the resulting fragment depend on the nucleotide sequence and the DNA source.

Each primer has different fragments so that just one base pair difference can prevent amplification, then it will produce polymorphs with different fragments and molecular weights.

Electrophoresis produces several band patterns in which the band pattern is divided into two categories: polymorphic bands and monomorphic bands. Polymorphic band is a DNA band that appears at the size of a bp (base pair) and a sample of certain fish, which in other fish samples is not found DNA band at that size. While monomorphic band show one shape and same sizes in same fish sample [22].

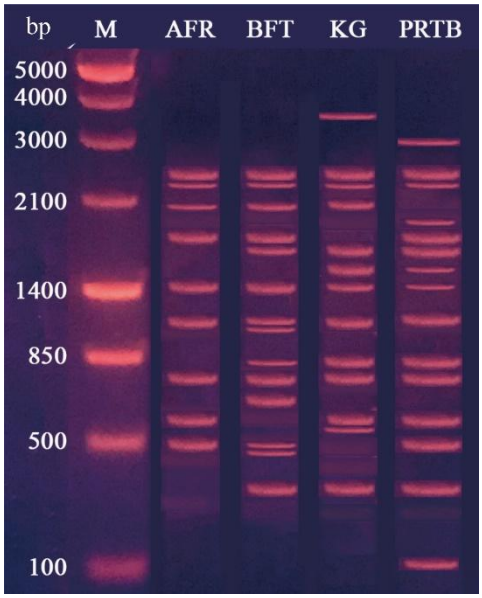

Fig. 2. Electrophoresis of DNA Amplification Result

Note: bp = base pair; M = Marker 1 Kb; AFP=Albino Full Red; BFT= Brazilian Fan Tail; KGT=Koi Guppy Tuxedo; PRTB=Platinum Red Tail Big Ears

Based on the amplification bands of the PCR results, it can be seen in Fig. 2 above using the OPA-03 primer to produce a total of 21 bands with the following divisions:

AFR : 9 monomorphic bands
BFT : 14 bands consisting; 11 monomorphic band, 3 polymorphic band
KGT : 13 bands consisting; 11 monomorphic band, 2 polymorphic band
PRTB : 15 bands consisting, 12 monomorphic bank, 3 polymorphic band

In the results of the study, there was only one strain that did not have a polymorphic band, namely the AFR strain, while the other three strains had a polymorphic band at a certain base pair size, the BFT strain at size 319.77; 308,631; and 300.68, KGT on size 352.58 and 304,292, and PRTB on size 350.17; 337,003 and 283,019. The strain that appears to have polymorphic bands indicate that the strain has genetic variation and has its uniqueness from other strains. Phenotypically, differences can be compared with body size, body color, fin shape, and fin color.

Table 1. DNA purity

No	Sample	Abs_{260}	Abs_{280}	Value of DNA Purity	KConcentration µg/ml
1	*A AFR*	0,1055	0,0571	1,85	263,7
2	*B BFT*	0,163	0,0847	1,92	407,5
3	*K KGT*	0,2226	0,1254	1,78	556,5
4	*P PRTB*	0,1487	0,0785	1,89	371,7

Note :AFP=Albino Full Red; BFT= Brazilian Fan Tail; KGT=Koi Guppy Tuxedo; PRTB=Platinum Red Tail Big Ears

Table 2. Polymorphic and monomorphic DNA bands

BP Position	AFR	BFT	KGT	PRTB
352.58			--*	
350.17				--*
344.377	--	--	--	--
342.859	--	--	--	--
339.971	--	--	--	
337.003				--*
334.778	--	--		--
332.059		--	--	--
328.915			--	--
325.877	--	--	--	--
321.241	--	--	--	--
319.77		--*		
315.678		--	--	--
312.525	--	--	--	--
308.631		--*		
306.591	--		--	--
304.292			--*	
302.285	--	--		--
300.68		--*		
295.16		--	--	--
283.019				--*

The BFT strain gives rise to three polymorphic bands with suspected variations in head color, body-color, and tail shape, color on the body of the BFT strain on the head is platinum with a slight red pattern on the back, body color mixed blue with black with a small stomach shape, and the shape of the BFT tail is a triangular fan (fantail) the shape looks elegant that resembles an open fan with a dorsal fin that extends also the color of the fin and red tail with the tip of the red tail. and vertical lines such as the skeleton of a fan's body are black. According to [23] This particular fin shape is called “Fantail” in Asia, marked by its broad vertical size, relatively short length, and rounded edges. According to [24], The Brazilian guppy was found in Upper Paraguay, Mato Grosso State, as a new strain in 2017.

The KGT strain has two polymorphic bands which are thought to have variations in fin color and body color. The koi guppy resembles a color scheme such as a red tail koi fish, white body, and red face, but this strain has a tuxedo color on the body which according to [2] guppy which has a half-light and half dark body color in Asia known as “tuxedo”, where the BFT strains display two contrasting colors between the front and back of their bodies. The front part of the body is lighter in color, while the lower part of the body is much darker, like the color scheme of the tuxedo.

Furthermore, the PRTB strain has three polymorphs with alleged variations in head color, body color, and fin shape. The color on the head of the body to the base of the tail is platinum with no additional markings and colors, while the color of the dorsal and caudal fins is a red color that is very true to its name, namely "red tail". While the term "big ears" in question is that the KGT has a large pectoral fin shape that looks like ears.

In the end, the single primer RAPD marker did not reveal all variation in genotype or phenotype. Suggestions for further research can be sequencing or using more primers or variations.

4.3 Genetic Relationship Analysis

The next step is to process the data using the NTSYS-PC program, which would produce a kinship tree or dendrogram tree. The dendrogram tree describes the kinship between a fish where the fish still have a close kinship. A dendrogram tree describes the level of closeness or kinship of fish, which is indicated by a percentage. The percentage is obtained based on the results of calculations through a comparison of the morphological characters possessed by each fish [25].

The dendrogram tree generated from four strains using OPA-03 primer has two divisions. The first divisions with a genetic similarity value of 69.5%, namely AFR and BFT, the second divisions has a genetic similarity of 71.5%, namely KGT and PRTB. Meanwhile, the genetic similarity between these two large groups is 66.5% because these four guppy fish strains are still in the same species.

The dendrogram tree illustrates that branch length indicates genetic distance. The shorter the branch, the closer the distance genetics and the longer the branch, the more distant the relationship kinship [26].

The first group AFR and BFT had the same genetic similarity of 69.5%. The similarity between these two strains is that they phenotypically have a compressed body shape and come from the same cultivator, namely Cilengkrang, Bandung. According to [27] cultivation environmental factors (stocking density, age, temperature, water quality, biology and physiology, diet, and maintenance) greatly affect the phenotype of an individual or population.

The difference in this group is 30.5%, AFR includes Albino strain guppy fish, where the body cannot produce black or dark pigments [2, 28] so as the name implies, the whole body is red, the tail is triangular (delta tail), has eye color Real Red Eye Albino (RREA). While the body BFT is dark blue while the tail and fins are red, the tail is round-tail and the eyes are black [29].

The second group between KGT and PRTB had a genetic similarity of 71.5%. The similarities between these two strains are phenotypically they have the same tail shape, namely a round tail. According to [29] around tail is the shape of the tail which the edges of the fishtail fin are curved or rounded. Another similarity, these two fish have the same tail color which is red while the body part is not red. Both of these strains have the same black eye color.

The difference between these two groups is 28.5% where the KGT as the name implies has a tuxedo pattern which according to [30], in the Asian region the name Tuxedo is given because the guppy has a half-black color on its body. While PRTB has iridophores pigment and the platinum color is located on the X-chromosome, and is on the Y-chromosome for the allele version in wild fish [23]. The two strains came from different cultivators. KGT came from Tangerang while PRTB came from Cilengkrang, Bandung.

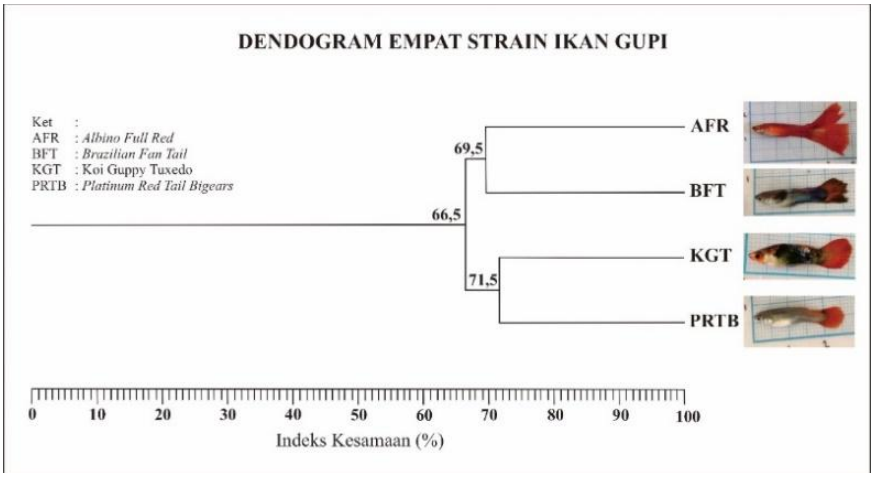

Fig. 3. Phylogenetic tree of four guppy strains with primary OPA-03

5. CONCLUSIONS

The first group, namely (AFR) Albino Full Red and (BFT) Brazilian Fan Tail, had a genetic similarity index in the range of 69.5%. The second group, namely (KGT) Koi Guppy Tuxedo and (PRTB) Platinum Red Tail Big Ears, had a genetic similarity index of 71.5%. Primer OPA-03 used in this study was good for analyzing the relationship between four strains of guppy fish. This can be seen from the number of ribbons that are visualized and analyzed for their level of relationship.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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